

The Figures:

Please substitute attached Figures 1-7 set forth on drawing sheets 1-7, respectively, for original Figures 1-7.

REMARKS

Claims 1-2, 4-5, and 7-33 are pending in this application. Claims 3 and 6 have been canceled without prejudice or disclaimer.

Claims 1, 4-5, 8, 28-29, and 31 have been amended. More specifically, claim 1 has been amended to incorporate the limitations of canceled claims 3 and 6. Claim 1 has also been amended to delete the term "fragment" and to correct minor grammatical and typographical errors. Support for claim 1 as amended appears throughout the specification and claims as originally filed. No new matter has been added.

Claims 4 and 5 have been amended to make them each properly dependent on claim 1. No new matter has been added.

Claim 8 has been amended to replace the term "such as" with the term "including." No new matter has been added.

Claim 28 has been amended to recite that the "transformant as set forth in claim 27, which is deposited under (Accession No: KCTC 10320BP)." Claim 29 has been amended to recite that the "transformant as set forth in claim 27, which is deposited under (Accession No: KCTC 10321BP)." Support for claims 28 and 29 appears throughout the specification and claims as originally filed. No new matter has been added.

Claim 31 has been amended to delete the term "the proper." No new matter has been added.

Submitted herewith is a Declaration UNDER 37 CFR § 1.108, by Eui-Sung CHOI, attesting that the transformants, described in the present specification,

were deposited under the terms of the Budapest Treaty. Also submitted is a Deposit Receipt for each deposit.

Also submitted herewith are substitute drawing sheets 1-7 containing Figures 1-7, respectively. The Figures have been amended as appropriate to incorporate Seq. ID Nos. No new matter has been added.

In addition, Applicants amended the Sequence Listing in order to incorporate the sequence information described in Fig. 2 into Sequence Listing. Particularly, applicants amended SEQ ID NOs: 9 and 10 because the amino acid sequences lack GS at C-terminus and added SEQ ID NO: 19 drawn to LipWt. The amendments and sequence listing do not introduce any new subject matter within the meaning of 35 U.S.C. §132. The submission, filed herewith in accordance with the relevant sections of 37 CFR 1.821, does not include new matter; and the content of the attached paper copy and the attached computer readable copy of the Sequence Listing are the same. Therefore, entry of the amendment is respectfully requested.

Also filed herewith is an Information Disclosure Statement citing two (2) references.

In view of the remarks set forth herein, further and favorable consideration is respectfully requested.

- I. At page 2, of the Official Action, the Examiner acknowledges receipt of Korean application 10-2220-0055575 filed on September 13, 2002; However, the Examiner notes that no English translation has been filed.***

Applicants submit herewith to the United States Patent and Trademark Office, a certified English translation of the priority document, Korean application 10-2220-0055575, along with a verified statement by Korean patent attorney Yun Ho HAN stating that the translation is a true and complete English translation of the subject Korean Patent Application filed on September 13, 2002.

- II. At page 2, of the Official Action, the Examiner notes that Fig. 2 contains amino acid sequences that need sequence identifiers.***

Accordingly, and as discussed above, Fig. 2 and the Sequence Listing have been amended to comply with the Examiners request. Thus, the Examiner is respectfully requested to withdraw this objection.

- III. At page 3, of the Official Action, claim 1 has been objected to as improperly reciting the term "in" instead of "on."***

Claim 1 has been amended to properly recite the term "on" in place of the improperly recited term "in." Accordingly, the Examiner is respectfully requested to withdraw this objection.

IV. At page 3, of the Official Action, claims 28 and 29 have been rejected under 35 USC § 112, first paragraph, as not meeting the written description requirement.

The Examiner asserts that claims 28 and 29 contain subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. The Examiner questions whether the present transformants were deposited under the terms of the Budapest treaty.

In view of the following, this rejection is respectfully traversed.

Attached hereto, please find a Declaration of Depositor dated November 18, 2007, by Eui-Sung CHOI, attesting to the fact that the subject deposits were made with the Korean Collection for Type Cultures as the International Depository Authority, in compliance with the Budapest Treaty, as Deposit Accession Numbers KCTC 10320BP and KCTC 10321BP. Claim 7 has been canceled without prejudice or disclaimer. Please see the attached Declaration. Also submitted is a copy of a Deposit Receipt for each Deposit.

In view of the Declaration and Deposit Receipts submitted herewith, Applicants assert that the present claims meet the written description requirement of 35 USC § 112. Accordingly, the Examiner is respectfully requested to withdraw this rejection.

IV. At page 4, of the Official Action, claims 1-33 have been rejected under 35 USC § 112, second paragraph, as being indefinite.

The Examiner asserts that claim 1 lacks antecedent basis for the limitation "the surface display vector fragment" in line 9; that it is unclear what is meant by a "fragment"; that it is unclear what is meant by "evolved activity"; that the phrase "such as" renders the claim indefinite; in claims 28 and 29, it is unclear what accession number is recited; and that the term "proper" in claim 31 is indefinite.

In view of the following, this rejection is respectfully traversed.

Claim 1 has been amended to incorporate the limitations of canceled claims 3 and 6, to delete the term "fragment," to replace the term "evolved" with the term "improved," and to correct several minor typographical and grammatical errors. No new matter has been added.

Claim 8 has been amended to replace the term "such as" with the term "including." No new matter has been added.

Claims 28 and 29 have been amended to delete the term "the proper." No new matter has been added.

In view of the foregoing, it is submitted that claims 1-2, 4-5, and 7-33 are clear and definite within the meaning of 35 USC § 112, second paragraph. Thus, the Examiner is respectfully requested to withdraw this rejection.

V. At page 6, of the Official Action, claims 1, 3-6, and 8, 9, have been rejected under 35 USC § 103(a) as unpatentable over US 6,020,180 in view of Kim et al.

The Examiner asserts the Applicant cannot rely upon the foreign priority papers to overcome this rejection because a translation of the papers has not

been made of record in accordance with 37 CFR § 1.55. As discussed hereinabove, a certified translation of the Korean priority document has been filed herewith.

Because Applicants are entitled to their priority date of September 13, 2002 and Kim et al. was published after this date, Kim et al. does not constitute prior art against the present claims. Accordingly, only the '180 patent will be discussed below.

In addition, the Examiner asserts that the '180 patent does not teach a CWP1, GAS1, or TIP1 surface display mediating gene, or a surface display vector, and does not teach a MOX or GAPDH promoter. The Examiner asserts that it would have been obvious to combine the teachings of the '180 patent on a method for identifying lipase variants with improved properties with Kim et al. who teach a cell surface display vector including a MOX or GAPDH promoter, an alpha-amylase signal sequence, an a CWP1, GAS1 or TIP1 surface display mediating gene, because Kim et al. state that a surface display system in *Hansenula* can provide a valuable tool for a screening system for useful protein ligand. Thus, it would have been obvious to the skilled artisan to use the cell surface display system because the screening system is useful for design of various biocatalysts to perform bioprocesses.

In view of the following, this rejection is respectfully traversed.

Claims 3 and 6 have been canceled without prejudice or disclaimer, and as to these claims, this rejection is moot. Claim 1 has been amended to incorporate the limitations of canceled claims 3 and 6.

As discussed, claim 1 has been amended to recite "[A] method for screening of a mutant lipase having an improved enzymatic activity comprising the following steps: 1) cloning a lipase gene into a surface display vector comprising a promoter gene, a gene coding a secretion signal sequence, a lipase gene or a mutant lipase gene, a surface display mediating gene selected from the group consisting of SED1, PIR2, TIP1, CWP1, GAS1 and WSC1, and a terminator gene; 2) preparing a mutant lipase gene library by mutagenic PCR using the lipase gene in the surface display vector of [[the]] step 1 as a template; 3) transforming the mutant lipase gene library of the step 2 and the surface display vector into host cells; and 4) measuring the activity of the mutant lipase displayed on the surface of the transformed host cell and selecting the lipase showing improved activity."

The U.S. Supreme Court in *Graham v. John Deere Co.*, 148 U.S.P.Q. 459 (1966) held that non-obviousness was determined under § 103 by (1) determining the scope and content of the prior art; (2) ascertaining the differences between the prior art and the claims at issue; (3) resolving the level of ordinary skill in the art; and, (4) inquiring as to any objective evidence of nonobviousness.

To establish a *prima facie* case of obviousness, the Examiner must establish: (1) that some suggestion or motivation to modify the references exists; (2) a reasonable expectation of success; and (3) that the prior art references teach or suggest all the claim limitations. *Amgen, Inc. v. Chugai Pharm. Co.*, 18 USPQ2d 1016, 1023 (Fed. Cir. 1991); *In re Fine*, 5 USPQ2d 1596, 1598 (Fed.

Cir. 1988); *In re Wilson*, 165 USPQ 494, 496 (C.C.P.A. 1970).

A *prima facie* case of obviousness must also include a showing of the reasons why it would be obvious to modify the references to produce the present invention. See *Ex parte Clapp*, 277 USPQ 972, 973 (Bd. Pat. App. & Inter. 1985). The Examiner bears the initial burden to provide some convincing line of reasoning as to why the artisan would have found the claimed invention to have been obvious in light of the teachings. *Id.* at 974.

A proper case of obviousness under 35 U.S.C. §103, requires that the prior art, as a whole, must suggest the desirability of making the claimed combination and provide a reasonable expectation of success. See *In re Dow Chemical Co.*, 837 F.2d 469, 5 USPQ2d 1529 (Fed. Cir. 1988).

The *Dow* court further held that “In determining whether such a suggestion can fairly be gleaned from the prior art, the full field of the invention must be considered for the person of ordinary skill is charged with knowledge of the entire body of technological literature, including that which might lead away from the claimed invention.” The court in *In re Gurley*, 27 F.3d 551 (Fed. Cir. 1994), held that “A prior art reference may be said to *teach away* when a person of ordinary skill, upon reading the reference, would be discouraged from following the path set out in the reference, or would be led in a direction divergent from the path that was taken by the applicant.” The court in *Busch & Lamb, Inc. v. Barnes-Hind/Hydro curve, Inc.*, 796 F.2d 443 (Fed. Cir. 1986), held that “A reference should be considered as a whole, and portions arguing against or teaching away from the claimed invention must be considered.”

Regarding motivation to modify a reference, the level of skill in the art cannot be relied upon to provide the suggestion to combine references. See *Al-Site Corp. v. VSI Int'l Inc.*, 174 F.3d 1308 (Fed. Cir. 1999). Although a prior art device "may be capable of being modified to run the way the apparatus is claimed, there must be a suggestion or motivation in the reference to do so." *In re Mills*, 916 F.2d 680 at 682.

If a proposed modification would render the prior art invention being modified unsatisfactorily for its intended purpose, then there is no suggestion or motivation to make the proposed modification. *In re Gordon*, 733 F.2d 900 (Fed. Cir. 1984). In addition, if a proposed modification or combination of prior art references would change the principle of operation of the prior art invention being modified, then the teachings of the references are not sufficient to render the claims *prima facie* obvious. *In re Ratti*, 270 F.2d 810, 813 (CCPA 1959).

Applicants submit that a *prima facie* case of obviousness has not been established because the '180 patent does not teach or suggest each and every claim limitation as required by *In re Wilson*.

More specifically, and as expressly admitted by the Examiner, "the '180 patent does not teach a CWP1, GAS1, or TIP1 surface display mediating gene, or a surface display vector, and does not teach a MOX or GAPDH promoter."

In addition, US 6,020,180 and Kim et al. neither teach nor suggest the claimed subject matter of amended claim 1. In particular, Kim et al. describes that HpCWP1p, HpGas1p, HpTip1p and HpSed1p derived from *Hansenula polymorpha* are GPI-anchor proteins and that the proteins act as surface

expression motifs and that a surface expression system is used for screening useful peptides and improved enzymes using flow cytometry. US 6,020,180 discloses lipase A derived from *Candida antartica*, a method for preparing muteins of the lipase and a method for analyzing enzymatic activity of the muteins. However, lipase A disclosed in US 6,020,180 is secreted into extracellular space. US 6,020,180 **neither teaches nor suggests** a method for expressing lipase on the surface of host cells using a surface expression system, as presently claimed. WO02/12509 (cited in the IDS filed herewith) which is related to Kim et al. describes that the surface expression system using surface proteins derived from *Hansenula polymorpha* such as HpCWP1p, HpGas1p, HpTip1p and HpSed1p can stably express proteins of interest onto the cell surface, thus finding numerous applications in various fields, including immobilization of biocatalysts and large-scale production of proteins, such as enzymes, antigens, antibodies, etc. Thus, WO02/12509 neither teaches nor suggests a surface expression system as a tool for screening a lipase having improved enzymatic activity. In the meantime, Shiraga *et al* (Journal of Molecular Catalysis B: Enzymatic (2002) 17: 167-173, June 7, 2002) (cited in the IDS filed herewith) identified by the Examiner of a related European application describes construction of a recombinatorial library of *Rhizopus oryzae* lipase (ROL) mutated in the lid domain on yeast cell surface and investigation of the effect of the amino acid sequence of the lid domain of on its substrate specificity. Particularly, the plasmid for the expression of ROL on the cell surface of *S. cerevisiae*, pWRS�17S, was constructed by subcloning the mutant ROL gene

fragment generated by combinatorial mutagenesis of the 6 amino acid on the lid domain into the plasmid comprising the genes encoding the GAPDH promoter, pre- α -factor leader sequence-encoding gene, C-terminal 320 amino acids of α -agglutinin and the terminator gene. The resulting expression plasmid is introduced into *E. coli* (This step is not clearly stated). However, that is a general recombinant DNA manipulation and the author of the Shiraga et al. mentioned the use of *E. coli* for a host for recombinant DNA manipulation at pp168, 2.1. After culturing *E. coli*, the plasmid for mutated ROLs was harvested and was used to transform the yeast. A library of ROL with the combinatorial mutagenesis of the lid domain was screened by halo assay. Shiraga et al. neither teaches nor suggests HpCWP1p, HpGas1p, HpTip1p and HpSed1p as surface expression mediators. In addition, Shiraga et al. never teaches nor suggests *in vivo* recombination performed in yeast which is used in the presently claimed subject matter (See step 3 of claim 1) rather than additional manipulation with *E. coli*. Thus, the subject matter of claim 1 is not obvious from any of the applied prior art or references cited in the Information Disclosure Statement filed herewith.

Lastly, comparing to the results between the prior art including US 6,020,180 and Shiraga et al. with the presently claimed subject matter, the presently claimed subject matter exhibits remarkable effects.

US 6,020,180 failed to obtain improved mutant enzymes using the methods described therein. In addition, although Shiraga et al. screened 20,000 clones, they did not obtain mutants with a higher activity than wild type enzyme

(pp171, right column). However, the present inventors screened 7,000 clones and obtained 3 clones, which exhibit more than 5 times increased lipase activity. Moreover the method of the present invention allows the easy screening of positive clones having improved specific activity.

The inventors hypothesized that surface expression vector of the lipase enables us to screen positive clones having increased specific activity more easily than the vector designed for the secretion of the gene product to the media does. In case of using the vector designed for the secretion, many factors, like an increase of transcription, translation, secretion, can affect on the halo size. As for the surface displayed lipase, a limited amount of protein is translocated onto the surface, making the secretion levels even. Therefore, the method of the present invention has an advantage to lower the possibilities to get false positive clone. The Examples 6 and 7 have demonstrated this advantage.

The inventors finally screened the clones having high lipase activity, which is CalB10 (L278P) and CalB14 (L219Q, L278P). L278P results in an increase of specific activity and L219Q results in an increase of the amount of the secreted lipase. Interestingly, 5 times increased lipase activity was observed in two kinds of mutants, when they displayed on the cell surface. However, when the lipase was secreted out of cells, the activity in the culture supernatant was confirmed to be increased 5 times (Lip10) and 10 times (Lip14). According to the experiment, the method of the present invention is very useful for screening mutants having high specific activity.

As described above, the claimed subject matter is distinguishable from the applied and/or discussed references including those submitted herewith in an Information Disclosure Statement. In addition, as discussed above, the presently claimed subject matter has unexpected remarkable effect over the prior art.

Accordingly, it is submitted that nothing in the '180 patent, or the '180 patent taken in combination with any discussed reference, teaches or suggests the subject matter of claims 1, 4-5, and 8-9, as required for obviousness under 35 USC § 103. Thus, the Examiner is respectfully requested to withdraw this rejection.

VI. At page 8, of the Official Action, claim 2, has been rejected under 35 USC § 103(a) as unpatentable over US 6,020,180 in view of Kim et al. and in further view of Uppenberg et al.

The Examiner asserts that it is unclear what is meant by a method "containing."

Claim 2 is dependent on amended claim 1. Again, as expressly admitted by the Examiner, "the '180 patent does not teach a CWP1, GAS1, or TIP1 surface display mediating gene, or a surface display vector, and does not teach a MOX or GAPDH promoter." Uppenberg et al. does not cure the deficiencies of the '180 patent because Uppenberg et al. also does not teach or suggest a CWP1, GAS1, or TIP1 surface display mediating gene, or a surface display vector, and does not teach a MOX or GAPDH promoter.

Further, none of the above discussed references render the claimed invention obvious. Please see the remarks and discussion set forth above and incorporated herein by reference.

Accordingly, it is submitted that nothing in the '180 patent and Uppenberg et al., taken alone or together, teaches or suggests the subject matter of claim 2 as required for obviousness under 35 USC § 103. Thus, the Examiner is respectfully requested to withdraw this rejection.

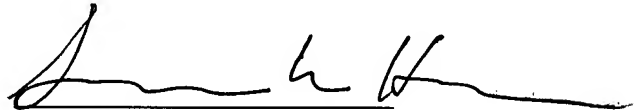
CONCLUSION

In view of the foregoing, Applicant submits that the pending claims are in condition for allowance. Early notice to that effect is earnestly solicited. The Examiner is invited to contact the undersigned attorney if it is believed that such contact will expedite the prosecution of the application.

In the event this paper is not timely filed, Applicant petitions for an appropriate extension of time. Please charge any fee deficiency or credit any overpayment to Deposit Account No. 14-0112.

Respectfully submitted,

THE NATH LAW GROUP

A handwritten signature in black ink, appearing to read 'Gary M. Nath', is written over a horizontal line.

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